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Purification and Characterization of a Major Cytoplasmic Antigen of *Candida albicans*

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In previous work (Jones, Infect. Immun. **30**:78-89, 1980) a major cytoplasmic antigen of *Candida albicans* was identified. In both humans and experimental animals, this antigen is released from *C. albicans* during the course of an invasive *C. albicans* infection and elicits a specific antibody response. In this study, we used diethylaminoethyl cellulose chromatography and concanavalin A-Sepharose chromatography to obtain purified preparations of the major cytoplasmic antigen from crude cytoplasmic extracts of *C. albicans*. Column chromatography yielded a purified preparation of the major cytoplasmic antigen, which produced a single line in polyacrylamide gel electrophoresis. Using crossed immunoelectrophoresis, we detected small concentrations of contaminating antigens in the purified preparation. We found that the major antigen was a single polypeptide chain containing about 435 amino acid residues and had a molecular weight of 54,300. This antigen did not possess any of 19 common enzymatic activities. Enzyme-linked immunosorbent assays are being developed to detect this antigen in serum and to detect antibody against the antigen.

Severe *Candida* infections are major causes of morbidity and mortality in immunocompromised patients (4). However, despite increasing clinical awareness of this problem, the diagnosis of deep-seated *Candida* infections remains a great challenge (4, 10, 13). Numerous investigators have described serological tests which are used to facilitate the diagnosis of *Candida* infections. One of the most widely studied serodiagnostic techniques is based on the detection of antibodies to unidentified components in homogenates of *Candida albicans*. Such homogenates contain significant amounts of cell wall mannan (9, 18, 25, 43), as well as a complex array of cytoplasmic antigens (1). Stallybrass (38) and Taschdjian et al. (42) were among the first to use such homogenates to test for precipitating antibodies in human sera by agar gel diffusion. Other techniques for measuring antibodies to such cytoplasmic antigens have also been developed; these include a latex agglutination test (40), counterimmunoelectrophoresis (35), crossed immunoelectrophoresis (1), a radioimmunoassay (18), and quantitative immunofluorescence (6). Recently, a radioimmunoassay for detecting the cytoplasmic antigens has been described (39). Conflicting conclusions concerning the diagnostic sensitivities and specificities of these techniques as applied to human sera have appeared (3, 7, 9-12, 14, 17, 21, 27, 28, 33, 34, 43). Explanations for this controversy emphasize differences between various antigen preparations, testing methods, and patient populations. Several authors (2, 6) have proposed that production of a well-characterized cytoplasmic antigen of *Candida* would be a major step toward development of a diagnostic test with good sensitivity, specificity, and reproducibility.

Previously, Jones described a cytoplasmic extract of *C. albicans* and an ammonium sulfate-soluble fraction of this extract (18). The sulfate-soluble fraction was found to be enriched for a major cytoplasmic antigen of *C. albicans*. Jones developed a radioimmunoassay in which ^{125}I-labeled sulfate-soluble fraction was used to measure antibody that was directed primarily against this major antigen (18). This technique showed that a major antigen released from *C. albicans* in experimentally infected rabbits or infected humans elicited a specific antibody response (19). Thus, it is clear that a purified preparation of the major cytoplasmic antigen should enable the development of immunoassays which are truly specific for the detection of antibody against this antigen.

In this paper we describe a method for producing such a purified major cytoplasmic antigen preparation. We analyzed the purity of this preparation by biochemical and immunological methods and characterized the antigen further.

MATERIALS AND METHODS

Source and culture of organism. Cultures of *C. albicans* type A strain B311 were obtained as previously described (18). A seed culture was inoculated into 100 liters of Sabouraud dextrose broth (Difco Laboratories, Detroit, Mich.) in a 200-liter fermentor. After incubation at 30°C for 18 h with constant stirring and injection of room air, the cells were harvested with a Sharples centrifuge, washed with distilled water, and again collected by centrifugation. The resulting paste was stored at −60°C.

Preparation of antigens. The cells were fractured by passage through a French press at 700 to 1,060 kg/cm^2, and the supernatant crude extract was separated from the cell walls by centrifugation at 12,000 × *g* for 30 min. Unless otherwise stated, this crude extract was used for the chromatographic purification described below.

For other experiments, a cytoplasmic extract and an ammonium sulfate-soluble fraction of this extract were prepared as previously described (18). The cell walls were used to produce cell wall mannan by the method of Peat et al. (32), with the modifications described by Sakaguchi et al. (36).

A series of experiments were performed to determine the optimal conditions for the purification of the major cytoplasmic antigen by diethylaminoethyl (DEAE)-cellulose (Sigma Chemical Co., St. Louis, Mo.) column chromatography. In the first experiment, a 200-ml DEAE-cellulose column was used. In all subsequent experiments, a cylindrical glass column (inside dimensions, 39 by 7 cm) filled with 1 liter (wet volume) of DEAE-cellulose was used. DEAE-cellulose chromatography was conducted at room temperature at a flow rate of approximately 200 ml/h. The ultraviolet transmittance of the column eluate was measured continuously by a model 8300 Uvicord II column recorder system (LKB, Bromma, Sweden). Pools of column eluate were concentrated by membrane ultrafiltration (Diaflo Ultrafilters; type YM 5000; Amicon Corp., Lexington, Mass.), extensively dialyzed against distilled deionized water, lyophilized, and stored at −20°C. The material obtained by DEAE-cellulose chromatography which contained predominantly the major cytoplasmic antigen was subjected to further purification to remove contaminating cell wall mannan by affinity chromatography on concanavalin A linked to Sepharose by an adaptation of the method of Ellsworth et al. (5). The solution containing material that did not bind to concanavalin A-Sepharose was concentrated, dialyzed, lyophilized, and stored as described above. The resulting material is referred to below as purified major antigen.

Production of antisera. Antisera to cell wall mannan and cytoplasmic extract were produced as previously described (18). Antiserum to purified major cytoplasmic antigen was produced in New Zealand white rabbits by two biweekly intramuscular injections of 0.5 mg of major antigen in 0.5 ml of Freund complete adjuvant, followed by biweekly intramuscular administration of 0.1 mg of purified major antigen in 0.5 ml of Freund incomplete adjuvant.

For the enzyme-linked immunosorbent assay, an immunoglobulin G fraction of anti-mannan serum was prepared by Na_2SO_4 precipitation and DEAE-cellulose chromatography (18).

PAGE. Horizontal thin-layer polyacrylamide gel electrophoresis (PAGE) was performed by the method of Fehrnström and Moberg (8). A single-phase gel containing 3.5% polyacrylamide in tris(hydroxymethyl)aminomethane (Tris)-glycine buffer was used.

Sodium dodecyl sulfate (SDS)-PAGE was performed by a modification of the method of Laemmli (23). Horizontal thin-layer gels containing 3% polyacrylamide (stacking gel) and 10% polyacrylamide (separation gel) in Tris-glycine buffer containing 0.1% SDS were used. The molecular weight markers (Dalton Mark VI; Sigma Chemical Co.) used were bovine plasma albumin (molecular weight, 66,000), ovalbumin (45,000), pepsin (34,700), trypsinogen (24,000), β-lactoglobulin (18,400), and lysozyme (14,300). The molecular weights of test materials were calculated by comparing their electrophoretic mobilities with the mobilities of the molecular weight marker proteins.

Immunoelectrophoresis. Immunoelectrophoresis was performed by the method of Weeke (45), using glass plates (9.4 by 8.3 cm) and 1% agarose (type I; Sigma Chemical Co.) in barbital buffer (pH 8.6; μ = 0.02). Fused rocket immunoelectrophoresis, crossed immunoelectrophoresis, and crossed-line immunoelectrophoresis were performed by modifications of the techniques of Svendsen (41), Weeke (46) and Guinet and Gabriel (15), and Kroll (22), respectively.

Compositions of antigen preparations. The protein concentrations of various antigen preparations were determined by the Folin phenol method, using bovine serum albumin as the standard (26). Carbohydrate concentrations were determined by the orcinol method, using mannose as the standard (20).

To detect and quantitate cell wall mannan in antigen preparations, we used a double antibody sandwich enzyme-linked immunosorbent assay similar to that described by Harding et al. (16). Plastic microtiter plate (Flow Laboratories, Hamden, Conn.) wells were each coated with 5 μg of rabbit anti-cell wall mannan immunoglobulin G diluted in 100 μl of phosphate-buffered saline (0.02 M sodium phosphate, 0.14 M NaCl, pH 7.5) by overnight incubation at 37°C. The plates were flooded with wash solution (0.005 M sodium phosphate, 0.14 M NaCl, 0.2% bovine serum albumin, 0.05% polyoxyethylene sorbitan monolaurate, pH 7.5) and aspirated dry. Serial twofold dilutions of a standard cell wall mannan solution or the sample being analyzed in 50-μl portions of phosphate-buffered saline containing 0.02% polyoxyethylene sorbitan monolaurate were added to the wells of the plates. The plates were incubated at 37°C for 30 min, allowed to stand at room temperature for 90 min, flooded with wash solution, and aspirated dry; 50 μl of phosphate-buffered saline containing rabbit anti-mannan immunoglobulin conjugated with horseradish peroxidase (type VI; Sigma Chemical Co.) by the periodate method (47) was added to each well. The optimal dilution of each batch of conjugated antibody was determined by preliminary experiments (44). The plates were again incubated at 37°C for 30 min, allowed to stand at room temperature for 30 min, washed, and aspirated dry. A 100-μl portion of 2,2′-

azino-di-3-ethylbenzthiazolinesulfonic acid (Sigma Chemical Co.) enzyme substrate solution (0.4 M 2,2'-azino-di-3-ethylbenzthiazolinesulfonic acid and 0.5 M H_2O_2 in 0.05 M sodium citrate, pH 4.0) was added, and the reaction was allowed to proceed to good color development at room temperature and then stopped by adding 100 μl of a solution containing 0.001 M edetic acid and 0.1 M hydrofluoric acid in 0.01 M NaOH (37). The optical density of each sample was then determined at 414 nm with a microsampling spectrophotometer (model 300-N; Gilford Instrument Laboratories, Inc., Oberlin, Ohio). The concentration of mannan in the sample was calculated by comparison with the optical densities of the serial dilutions of the mannan standard.

The cytoplasmic extract and purified major antigen were tested semiquantitatively for 19 enzyme activities by using a commercially available enzyme test system (API ZYM enzyme substrate system; Analytab Products, Plainview, N.Y.).

An amino acid analysis of purified major antigen was performed at the Amino Acid Analyzer Facility, Biophysics Department, University of Wisconsin, Madison. After hydrolysis (29), the amino acid composition was determined by using a standard automated technique (24). The number of moles of each amino acid per mole of purified major antigen protein was calculated, based on a molecular weight range of major antigen determined by SDS-PAGE. As the tryptophan content could not be determined by the method used, we assumed for these calculations that no tryptophan was present.

RESULTS

Determining conditions for purification of the major antigen. Since the major antigen had a less negative charge than most other cellular components, as judged by PAGE (18), we performed experiments to determine the optimal conditions for purification by using DEAE-cellulose (positively charged solid-phase) chromatography.

In the first experiment, cytoplasmic extract was loaded onto a 200-ml DEAE-cellulose column equilibrated with 0.067 M Tris (Trizma Base; Sigma Chemical Co.) titrated to pH 7.5 with HCl (Tris buffer; ionic strength, 0.10). After a peak of ultraviolet-absorbing material eluted from the column, the material which remained attached to the DEAE-cellulose was eluted with Tris buffer containing a continuously increasing concentration gradient of NaCl (Fig. 1A). Individual fractions of eluate were then tested by fused rocket immunoelectrophoresis against rabbit anti-cytoplasmic extract serum. A prominent precipitin arc was produced by the fractions collected from the point just after the beginning of the NaCl gradient through the point at which the eluant was Tris buffer containing 0.04 M NaCl (ionic strength, 0.14). Several other antigens were detected in the fractions that were

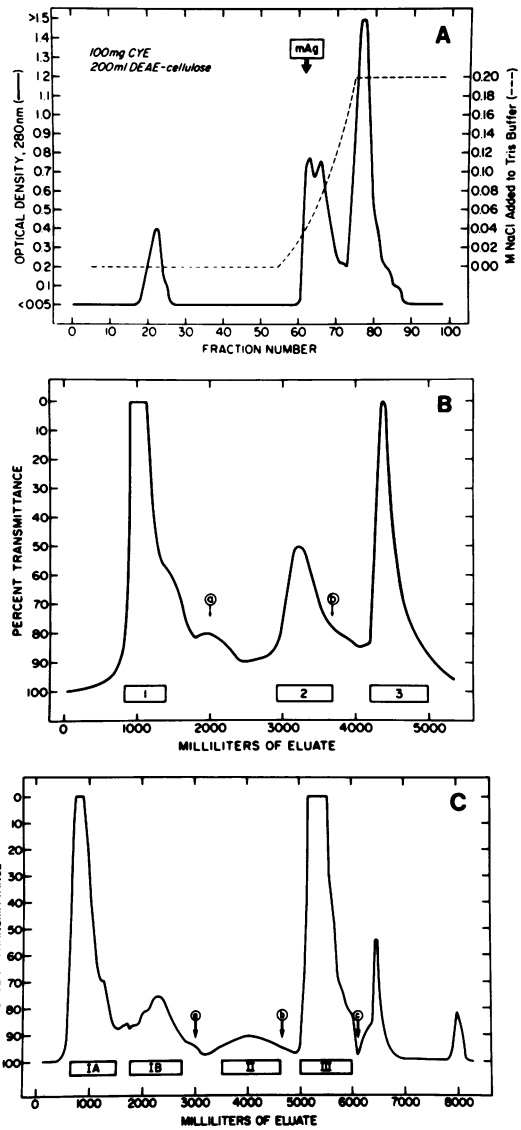

FIG. 1. *Elution patterns of DEAE-cellulose chromatography columns. (A) A total of 200 mg of cytoplasmic extract (CYE) was added to a column and then eluted with Tris buffer containing a continuous NaCl gradient. Each fraction contained 2 ml of eluate. mAg, Major antigen. (B) A total of 400 mg of crude Candida extract protein was added to a column and eluted initially with Tris buffer. Beginning at a, the eluant was Tris buffer containing 0.04 NaCl; beginning at b, the eluant was Tris buffer containing 0.2 M NaCl. (C) A total of 400 mg of crude Candida extract protein was added to a column and eluted initially with Tris buffer containing 0.01 M NaCl. Beginning at a, b, and c, the eluants were Tris buffer containing 0.04, 0.2, and 2 M NaCl, respectively. The numbered rectangles in (B) and (C) indicate the column pools collected.*

collected as the ionic strength was increased further. In a previous paper (18), a sulfate-soluble fraction was shown to be enriched for the major cytoplasmic antigen. When the sulfate-soluble fraction was studied by rocket immunoelectrophoresis against anti-cytoplasmic extract, it produced a number of precipitate peaks; the most prominent of these was the major antigen. When column fractions obtained by elution with buffers having ionic strengths of 0.10 to 0.14 were compared with the sulfate-soluble fraction by fused rocket immunoelectrophoresis, the most prominent precipitate peak in each fraction fused with the major antigen precipitate peak. Thus, as Fig. 1A shows, the major antigen was eluted from the DEAE-cellulose column which we used with eluants having ionic strengths of 0.10 to 0.14.

Therefore, a stepwise elution of the crude cytoplasmic extract from a 1-liter DEAE-cellulose column was performed (Fig. 1B). Pools of eluate were compared with cytoplasmic extract by PAGE (Fig. 2A) and with the sulfate-soluble fraction by fused rocket immunoelectrophoresis against rabbit anti-cytoplasmic extract. Both of these techniques showed that the eluate pool obtained with the Tris buffer containing 0.04 M NaCl (Fig. 1B, pool 2) was greatly enriched for major antigen and contained virtually all of the major antigen recoverable, although other components were also detected in this pool by PAGE. Crossed immunoelectrophoresis analyses of the cytoplasmic extract (Fig. 3A) and pool 2 (Fig. 3B) against anti-cytoplasmic extract revealed that there was a decrease from 33 or more precipitate peaks in the extract to 15 peaks in pool 2. Both the cytoplasmic extract and pool 2 produced a large precipitate peak which was visible before staining.

Preparation of purified major antigen. The components contaminating the major antigen in pool 2 had an electrophoretic mobility that was slightly less than the mobility of the major antigen, suggesting that these components would not be retained by the DEAE-cellulose if an initial column eluate having an ionic strength slightly greater than 0.10 were used. After a series of trials, we found that initial elution with Tris buffer containing 0.01 M NaCl followed by elution with Tris buffer containing 0.04 M NaCl (Fig. 1C) enabled us to collect a pool (pool II) which produced a single protein band during PAGE (Fig. 2B) in the position expected for the major antigen. Other pools also contained some of the major antigen.

A further analysis of pool II revealed that it contained approximately 50% protein and 50% carbohydrate including substantial amounts of mannan, as determined by an enzyme-linked immunosorbent assay. Therefore, the contaminating mannan was removed from pool II by affinity chromatography with concanavalin A-Sepharose. The yield of purified major antigen from pool II was 36.7% of its dry weight and represented 1.4% of the original protein content of the crude cytoplasmic extract.

Purified major antigen contained less than 5% carbohydrate. When a bovine serum albumin

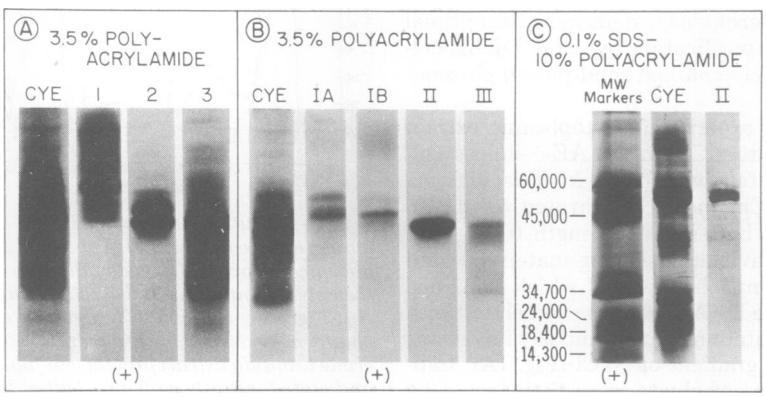

FIG. 2. *Patterns of protein-staining bands obtained by horizontal thin-layer PAGE and SDS-PAGE. The position of the anode is indicated by a plus sign. The gels were stained with Coomassie brilliant blue R-250. The dry weights of the materials applied to the tracks of the gels were as follows. (A) Comparison of a 200-μg sample of cytoplasmic extract (lane CYE) with 100-μg samples of pools 1, 2, and 3 (lanes 1, 2, and 3, respectively) obtained as described in the legend to Fig. 1. (B) Comparison of a 200-μg sample of cytoplasmic extract (lane CYE) with 100-μg samples of pools IA, IB, II, and III (lanes IA, IB, II, and III, respectively) obtained as described in the legend to Fig. 1. (C) Comparison of a 135-μg sample of proteins of known molecular weights (MW) with a 200-μg sample of cytoplasmic extract (lane CYE) and a 100-μg sample of pool II (lane II). A molecular weight scale appears at the left.*

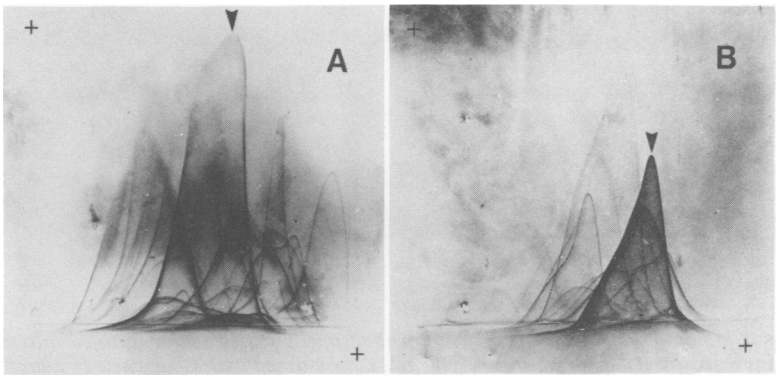

FIG. 3. *Crossed immunoelectrophoresis of cytoplasmic extract against rabbit anti-cytoplasmic extract antiserum (10 μl/cm²). The position of the anode in each dimension is indicated by a plus sign. The position of the major antigen is indicated by arrowheads. Gels were stained with Coomassie brilliant blue R-250. (A) Cytoplasmic extract protein (120 μg). First-dimension electrophoresis was conducted at 20 V/cm for 1 h, and second-dimension electrophoresis was conducted at 3.5 V/cm for 18 h. (B) Pool 2 protein (60 μg). First-dimension electrophoresis was conducted at 20 V/cm for 1 h, and second-dimension electrophoresis was conducted at 1.5 V/cm for 18 h.*

standard (15% N) was used, this antigen contained 9.4% N, and its mannan content was less than 6 ng/mg (dry weight), as determined by an enzyme-linked immunosorbent assay.

To confirm the identity of the major antigen with the prominent, densely staining precipitin peak (Fig. 3A, arrowhead), we performed crossed-line immunoelectrophoresis between the cytoplasmic extract and the purified major antigen preparation against anti-cytoplasmic extract (Fig. 4).

Detection of contaminants in purified major antigen. By crossed immunoelectrophoresis of the purified major antigen preparation against anti-cytoplasmic extract, it was possible to identify contaminants which were not detected by PAGE. Thus, in Fig. 5A, three to five small, faintly staining peaks are present in addition to the major antigen peak. Since these contaminants had electrophoretic mobilities very similar to the mobility of the major antigen and the areas under the precipitate peaks were proportional to the magnitude of the antigen-antibody reaction (45), we determined that the major antigen accounted for 95% of the antigenic reactivity of the purified preparation.

To detect contaminants in the purified major antigen preparation that might have been present in the cytoplasmic extract in such low concentrations that they failed to elicit a specific antibody response in animals immunized with the cytoplasmic extract, we produced a rabbit antiserum to the purified preparation. Crossed immunoelectrophoresis of the cytoplasmic extract against this antiserum (Fig. 5B) again produced the major antigen peak and three to five smaller, faintly staining precipitate peaks. It is possible that there were contaminants in the purified preparation which had electrophoretic mobilities similar to the mobility of the major antigen and were not antigenic.

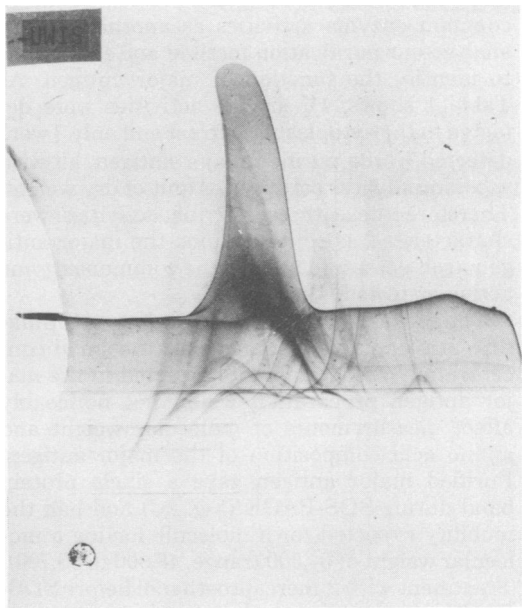

FIG. 4. *Crossed-line immunoelectrophoresis of 120 μg of cytoplasmic extract protein (well) and 45 μg of purified major cytoplasmic antigen (line) against rabbit anti-cytoplasmic extract antiserum (10 μl/cm²). First-dimension electrophoresis was conducted at 20 V/cm for 1 h, and second-dimension electrophoresis was conducted at 3 V/cm for 18 h.*

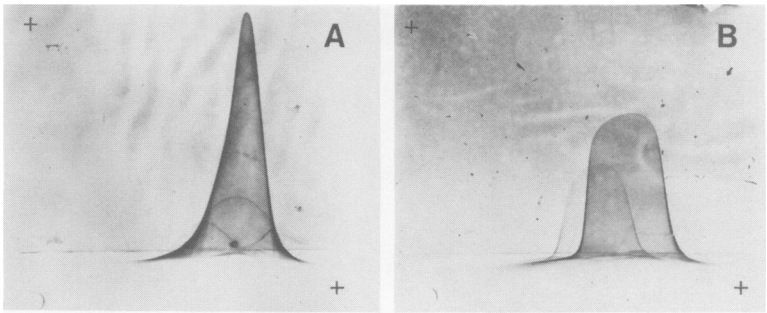

FIG. 5. *Crossed immunoelectrophoresis in which rabbit anti-purified major antigen antiserum was used. The plus signs indicate the electrode positions. The gels were stained with Coomassie brilliant blue R-250. (A) Crossed immunoelectrophoresis of 60 μg of pool II protein against rabbit anti-cytoplasmic extract antiserum (10 μl/cm²). First- and second-dimension electrophoreses were conducted at 20 V/cm for 1 h and 1.5 V/cm for 18 h, respectively. (B) Crossed immunoelectrophoresis of 120 μg of cytoplasmic extract protein against rabbit anti-purified major antigen antiserum (10 μl/cm²). First- and second-dimension electrophoreses were conducted at 20 V/cm for 1 h and 1 V/cm for 18 h, respectively.*

Enzymatic analysis of purified major antigen. Identical amounts of cytoplasmic extract and purified major antigen were tested for 19 common enzyme activities as another way to analyze our purification method and to attempt to identify the function of major antigen. As Table 1 shows, 11 enzyme activities were detected in the cytoplasmic extract and only 3 were detected in the purified major antigen, all with substantially less activity per unit of dry weight. Therefore these three enzyme activities were characterized as contaminants; the major antigen itself possessed none of the common enzyme activities tested.

Characterization of the major cytoplasmic antigen. We assumed that the small concentration of contaminants detected in the major antigen preparation would not noticeably affect measurements of molecular weight and amino acid composition of the major antigen. Purified major antigen gave a single protein band during SDS-PAGE (Fig. 2C) and had the mobility expected for a molecule having a molecular weight of 54,300 (range, 48,900 to 59,700). Treatment with 2-mercaptoethanol before SDS-PAGE did not change the electrophoretic mobility, indicating that the major antigen is a single polypeptide chain. An amino acid analysis (Table 2) indicated that the major antigen contained approximately 435 amino acid residues, including one cystine and four methionines.

TABLE 1. *Enzyme activities in cytoplasmic extract and purified major antigen*

Enzyme activity	Amt (nmol) of substrate consumed in 4 h with 30 μg of:	
	Cytoplasmic extract	Purified major antigen
Acid phosphatase	30	5
Alkaline phosphatase	≥40	10
Chymotrypsin	0	0
Cystine aminopeptidase	10	0
Esterase	0	0
Esterase lipase	5	0
α-Fucosidase	0	0
α-Galactosidase	0	0
β-Galactosidase	0	0
α-Glucosidase	20	0
β-Glucosidase	≥40	0
β-Glucuronidase	0	0
Leucine aminopeptidase	≥40	0
Lipase	0	0
α-Mannosidase	10	0
N-acetyl-β-glucosidase	5	0
Phosphoamidase	20	5
Trypsin	0	0
Valine aminopeptidase	10	0

DISCUSSION

Development of a procedure for obtaining a purified preparation of major antigen required consideration of three problems. First, since more than 30 protein components, many having charges and electrophoretic mobilities similar to those of the major antigen, were readily detectable in cytoplasmic extracts (Fig. 2 and 3A), it was clear that separation methods which depended upon differences in these properties would not produce perfect results. The second problem was the presence of cell wall mannan, a heterogenous collection of molecules with varying molecular weights and phosphate contents (31), in the cytoplasmic extract (18). The third problem was the development of a method suitable for large-scale production of the major antigen.

We performed experiments to devise a method

TABLE 2. *Amino acid analysis of major antigen in pool II*

Amino acid	No. of residues per mol[a]
Alanine	49 (44–54)
Arginine	12 (11–14)
Aspartic acid	52 (47–57)
Cysteine-cysteine	1 (1)
Glutamic acid	44 (40–49)
Glycine	40 (36–44)
Histidine	7 (6–8)
Isoleucine	23 (21–25)
Leucine	36 (32–39)
Lysine	34 (31–38)
Methionine	4 (4)
Phenylalanine	15 (14–17)
Proline	19 (17–21)
Serine	36 (32–39)
Threonine	26 (24–29)
Tyrosine	13 (12–14)
Valine	24 (21–26)

[a] Data are expressed as the number of moles of each residue per mole of major antigen protein, assuming a molecular weight of 54,300 for major antigen. The ranges of values are in parentheses and were calculated by using the lower limit (48,900) and upper limit (59,700) of major antigen molecular weight as determined by SDS-PAGE. Assuming that major antigen is a single polypeptide chain, the molecule contains 435 residues (range, 393 to 479).

to purify the major cytoplasmic antigen by stepwise elution of a crude cytoplasmic extract from a DEAE-cellulose column. We developed procedures for producing two different preparations. The first procedure (Fig. 1B) resulted in a preparation (pool 2) that contained a high yield of the major antigen and a number of other components as well (Fig. 3B). As determined by PAGE, the major contaminant in this preparation had slightly less electrophoretic mobility than the major antigen (Fig. 2A), and, fortunately, it was poorly immunogenic in rabbits, mice, and humans (18). The high yields of major antigen obtained by this method might make it more suitable for large-scale production.

The second purification method (Fig. 1C) resulted in the production of a material (pool II) which produced a single protein band in PAGE and SDS-PAGE analyses (Fig. 2B and C). Cell wall mannan contaminants in this preparation were removed by concanavalin A-Sepharose affinity chromatography. The resulting material was pure enough to permit initial characterization of the major antigen molecule, although small amounts of five other antigens were detected by crossed immunoelectrophoresis (Fig. 5A). We found that the major antigen is a single polypeptide chain which has a molecular weight of 54,300 (Fig. 2C) and is composed of approximately 435 amino acid residues and the equivalent of one cystine residue; thus, the molecule probably contains one intrachain disulfide bridge (Table 2). The major antigen contains 5% carbohydrate and stains with the periodic acid-Schiff reagent (18), thus indicating that it is a glycoprotein. It has been shown that this compound is not expressed on the surface of the organism (18) and that it does not contain any of 19 common enzymatic activities (Table 1). These observations and the high concentration of this antigen in cytoplasmic extracts suggest that it may be a structural component of the organism. Obviously, further studies will be needed to determine the precise function of this major cytoplasmic antigen.

In the future we intend to demonstrate that the major antigen from *C. albicans* serogroup A is also present in *C. albicans* serogroup B and in *Candida tropicalis*, another *Candida* species which is a major pathogen in compromised human hosts (48). Previously reported data have indicated that this compound is released in the course of deep-seated *Candida* infections and that it elicits a specific antibody response (18). We are presently developing enzyme-linked immunosorbent assays to detect this antigen and its antibodies in human and experimental animal sera. These enzyme-linked immunosorbent assays will be potentially useful tools for serodiagnosis of invasive candidiasis and for studies of the natural history of and immune response to this type of infection and as monitors of therapeutic efficacy. We are also studying the possible use of the major antigen in delayed-type hypersensitivity skin testing.

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LITERATURE CITED

1. **Axelsen, N. H.** 1973. Quantitative immunoelectrophoretic methods as tools for a polyvalent approach to standardization in the immunochemistry of *Candida albicans*. Infect. Immun. 7:949–960.
2. **Axelsen, N. H.** 1976. Analysis of human *Candida* precipitins by quantitative immunoelectrophoresis: a model for analysis of complex microbial antigen-antibody systems. Scand. J. Immunol. 5:177–190.
3. **Dee, T. H., and M. W. Rytel.** 1975. Clinical application of counterimmunoelectrophoresis in detection of *Candida* serum precipitins. J. Lab. Clin. Med. 85:161–166.
4. **Edwards, J. E., Jr., R. I. Lehrer, E. R. Stiehm, T. J. Fischer, and L. S. Young.** 1978. Severe candidal infections—clinical perspective, immune defense mechanisms, and current concepts of therapy. Ann. Intern. Med. 89:91–106.

5. **Ellsworth, J. H., E. Reiss, R. L. Bradley, H. Chmel, and D. Armstrong.** 1977. Comparative serological and cutaneous reactivity of candidal cytoplasmic proteins and mannan separated by affinity for concanavalin A. J. Clin. Microbiol. **5**:91–99.
6. **Estes, G., M. Munoz, N. Burdash, and G. Virella.** 1980. The serology of candidiasis: advantages of quantitative approaches using defined antigens. Clin. Immunol. Newslett. **1**:1–5.
7. **Everett, E. D., F. M. LaForce, and T. C. Eickhoff.** 1975. Serologic studies in suspected visceral candidiasis. Arch. Intern. Med. **135**:1075–1078.
8. **Fehrnström, H., and U. Moberg.** 1977. SDS and conventional polyacrylamide gel electrophoresis with LKB 2117 Multiphor. Application note 306. LKB-Produkter AB, Bromma, Sweden.
9. **Filice, G., B. Yu, and D. Armstrong.** 1977. Immunodiffusion and agglutination tests for *Candida* in patients with neoplastic disease: inconsistent correlation of results with invasive infections. J. Infect. Dis. **135**:349–357.
10. **Gaines, J. D., and J. S. Remington.** 1973. Diagnosis of deep infection with *Candida*: a study of *Candida* precipitins. Arch. Intern. Med. **132**:699–702.
11. **Gentry, L. O., T. R. McNitt, and L. Kaufman.** 1978. Use and value of serologic tests for the diagnosis of systemic candidiasis in cancer patients: a prospective study of 146 patients. Curr. Microbiol. **1**:239–242.
12. **Gleu, R. H., H. R. Buckley, H. M. Rosen, R. C. Moellering, Jr., and J. E. Fischer.** 1978. Serologic tests in the diagnosis of systemic candidiasis: enhanced diagnostic accuracy with crossed immunoelectrophoresis. Am. J. Med. **64**:586–591.
13. **Goldstein, E., and P. D. Hoeprich.** 1972. Problems in the diagnosis and treatment of systemic candidiasis. J. Infect. Dis. **125**:190–193.
14. **Guinan, M. E., M. R. Portas, and H. R. Hill.** 1979. The *Candida* precipitin test in an immunosuppressed population. Cancer (Philadelphia) **43**:299–302.
15. **Guinet, R. M. F., and S. M. Gabriel.** 1980. *Candida albicans* group A-specific soluble antigens demonstrated by quantitative immunoelectrophoresis. Infect. Immun. **29**:853–858.
16. **Harding, S. A., J. P. Brody, and D. E. Normansell.** 1980. Antigenemia detected by enzyme-linked immunosorbent assay in rabbits with systemic candidiasis. J. Lab. Clin. Med. **95**:959–966.
17. **Holder, I. A., P. J. Kozinn, and E. J. Law.** 1977. Evaluation of *Candida* precipitin and agglutination tests for the diagnosis of systemic candidiasis in burn patients. J. Clin. Microbiol. **6**:219–223.
18. **Jones, J. M.** 1980. Quantitation of antibody against cell wall mannan and a major cytoplasmic antigen of *Candida* in rabbits, mice, and humans. Infect. Immun. **30**: 78–89.
19. **Jones, J. M.** 1980. Kinetics of antibody responses to cell wall mannan and a major cytoplasmic antigen of *Candida albicans* in rabbits and humans. J. Lab. Clin. Med. **96**:845–860.
20. **Kabat, E. A., and M. M. Mayer.** 1967. Experimental immunochemistry. Charles C Thomas, Publisher, Springfield, Ill.
21. **Kozinn, P. J., R. S. Galen, C. L. Taschdjian, P. L. Goldberg, W. Protzman, and M. A. Kozinn.** 1976. The precipitin test in systemic candidiasis. J. Am. Med. Assoc. **235**:628–629.
22. **Kroll, J.** 1973. Crossed-line immunoelectrophoresis, p. 79–81. *In* N. H. Axelsen, J. Kroll, and B. Weeke (ed.), A manual of quantitative immunoelectrophoresis—methods and applications. Universitetsforlaget, Oslo, Norway.
23. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
24. **Lee, P. L.** 1974. Single-column system for accelerated amino acid analysis of physiological fluids using lithium buffers. Biochem. Med. **10**:107–121.
25. **Longbottom, J. L., W. O. Brighton, G. Edge, and J. Pepys.** 1976. Antibodies mediating type I skin test reactions to polysaccharide and protein antigens of *Candida albicans*. Clin. Allergy **6**:41–49.
26. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
27. **Marier, R., and V. T. Andriole.** 1978. Usefulness of serial antibody determinations in diagnosis of candidiasis as measured by discontinuous counterimmunoelectrophoresis using HS antigen. J. Clin. Microbiol. **8**:15–22.
28. **Merz, W. G., G. L. Evano, S. Shadomy, S. Anderson, L. Kaufman, P. J. Kozinn, D. W. MacKenzie, W. P. Protzman, and J. S. Remington.** 1977. Laboratory evaluation of serological tests for systemic candidiasis: a cooperative study. J. Clin. Microbiol. **5**:596–603.
29. **Moore, S.** 1972. Improved methods for automated amino acid analysis, p. 629–653. *In* Chemistry and biology of peptides. Proceedings of the Third American Peptide Symposium. Ann Arbor Science Publications, Ann Arbor, Mich.
30. **Odds, F. C.** 1979. *Candida* and candidiases. University Park Press, Baltimore.
31. **Okubo, Y., Y. Honma, and S. Suzuki.** 1979. Relationship between phosphate content and seriological activities of the mannans of *Candida albicans* strains NIH A-207, NIH B-792, and J-1012. J. Bacteriol. **137**:677–680.
32. **Peat, S., W. J. Whelan, and T. E. Edwards.** 1961. Polysaccharides of baker's yeast. IV. Mannan. J. Chem. Soc. **1**:29–34.
33. **Preisler, H. D., H. F. Hasenclever, and E. S. Henderson.** 1971. Anti-*Candida* antibodies in patients with acute leukemia: a prospective study. Am. J. Med. **51**: 352–361.
34. **Preisler, H. D., H. F. Hasenclever, A. A. Levetan, and E. S. Henderson.** 1969. Serologic diagnosis of disseminated candidiasis in patients with acute leukemia. Ann. Intern. Med. **70**:19–30.
35. **Remington, J. S., J. D. Gaines, and M. A. Gilmer.** 1972. Demonstration of *Candida* precipitins in human sera by counterimmunoelectrophoresis. Lancet **i**:413.
36. **Sakaguchi, V., S. Suzuki, M. Suzuki, and H. Sunayama.** 1967. Biochemical and immunochemical studies of fungi. VIII. Immunochemical studies of mannans of *Candida albicans* and *Saccharomyces cerevisiae*. Jpn. J. Microbiol. **11**:119–128.
37. **Saunders, G. C., E. H. Clinard, M. C. Bartlett, P. M. Petersen, W. M. Sanders, R. J. Payne, and E. Martinez.** 1978. Serologic test systems development. Los Alamos Scientific Laboratory Progress Report LA-7078-PR. United States Department of Energy, Washington, D.C.
38. **Stallybrass, F. C.** 1964. *Candida* precipitins. J. Pathol. Bacteriol. **87**:89–97.
39. **Stevens, P., S. Huang, L. S. Young, and M. Berdischewsky.** 1980. Detection of *Candida* antigenemia in human invasive candidiasis by a new solid phase radioimmunoassay. Infection **8**:S334–S338.
40. **Stickle, D., L. Kaufman, S. O. Blumer, and D. W. McLaughlin.** 1972. Comparison of a newly developed latex agglutination test and an immunodiffusion test in the diagnosis of systemic candidiasis. Appl. Microbiol. **23**:490–499.
41. **Svendsen, P. J.** 1973. Fused rocket immunoelectropho-

resis, p. 69–70. *In* N. H. Axelsen, J. Kroll, and B. Weeke (ed.), A manual of quantitative immunoelectrophoresis—methods and applications. Universitetsforlaget, Oslo, Norway.

42. **Taschdjian, C. L., G. B. Dobkin, L. Caroline, and P. J. Kozinn.** 1964. Immune studies relating to candidiasis: experimental and preliminary clinical studies on antibody formation in systemic candidiasis. Sabouraudia **3**:129–139.
43. **Taschdjian, C. L., M. S. Seelig, and P. J. Kozinn.** 1973. Serological diagnosis of candidal infections. Crit. Rev. Clin. Lab. Sci. **4**:19–59.
44. **Voller, A., D. E. Bedwell, and A. Bartlett.** 1979. The enzyme linked immunosorbent assay. Dynatech Europe, Guernsey, Great Britain.
45. **Weeke, B.** 1973. General remarks on principles, equipment, reagents, and procedures, p. 15–35. *In* N. H. Axelsen, J. Kroll, and B. Weeke (ed.), A manual of quantitative immunoelectrophoresis—methods and applications. Universitetsforlaget, Oslo, Norway.
46. **Weeke, B.** 1973. Crossed immunoelectrophoresis, p. 47–56. *In* N. H. Axelsen, J. Kroll, and B. Weeke (ed.), A manual of quantitative immunoelectrophoresis—methods and applications. Universitetsforlaget, Oslo, Norway.
47. **Wilson, J., and P. K. Nakane.** 1978. Production of enzyme-antibody conjugates, p. 215–226. *In* W. Knapp (ed.), Immunofluorescence and related techniques. Elsevier/North Holland Publishing Co., Amsterdam.
48. **Wingard, J. R., W. G. Merz, and R. Saral.** 1979. *Candida tropicalis*: a major pathogen in immunocompromised patients. Ann. Intern. Med. **91**:539–543.